The Preparation of Absolute Alcohol for Histological and Chemical Purposes.*

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A cheap and convenient method of preparing absolute alcohol for histological purposes on a small scale in a school or college is much needed. The ordinary methods given in text-books of organic chemistry require a considerable amount of apparatus and many different appliances which an ordinary biological laboratory might not conveniently have, and it is because of this fact that the following method has been devised.

Ordinary commercial alcohol about 90 to 95 per cent. strong is placed in a bottle or flask and small quantities of calcium carbide added at different intervals of time dependant on the rapidity with which the carbide is attacked,—about once in six hours is sufficient; if the mixture be placed in a warm place, however, the action is much more rapid. 125 grams may be added at the beginning of the operation, and 25 grams at a time every 5 or 6 hours thereafter until the reaction is finished. This point may be recognized both by the cessation of the appearance of bubbles of acetylene, or from the fact that the freshly added carbide remains several hours unacted upon.

The carbide acts upon the water in the alcohol, forming slaked lime and acetylene. (Reaction) $CaC_2+2H_2O=Ca(OH)_2+C_2H_2$. When it is dehydrated the action ceases. During the process the mixture should not be shaken or agitated, because the slag or residue of slaked lime becomes suspended in the liquid and settles on the added portions of carbide thus preventing further action. If the carbide is added at different intervals it spreads out upon the spent residue forming a layer that will be acted upon.

When the last layer of carbide remains unchanged after being in contact with the alcohol for some hours, the action is considered complete. If much matter remains suspended in the liquid, the mixture is allowed to stand a little longer till the supernatant liquid is clear and well settled. The alcohol is now carefully decanted or siphoned into a dry bottle and kept therein until used.

* Extract from thesis for the Bachelor's degree in Science. I wish to thank my professor, Dr. J. A. Nieuwland, who suggested the subject of this thesis.

Alcohol prepared in this manner is completely dehydrated but contains as an impurity acetylene gas, some lime in solution, also some traces of organic sulphur compounds. The alcohol though clear may be of a strong yellow color due to these compounds especially so when denatured alcohol is used. This color, however, is not objectionable when the primary end is to obtain an absolute alcohol for histological purposes. The acetylene may be almost entirely removed by warming the liquid to about 75° C. This may be easily accomplished by placing the bottle on a steam radiator for two or three hours till the heat drives the gas out of solution. The acetylene that remains does not affect the reagents nor mounts. The lime disolved is not appreciable, being about 1 part by weight in 30,000 of alcohol by volume. Though this alcohol is not strictly pure, the presence of lime is not deleterious, but on the contrary is beneficial. The alkali appears to fix the dye into the section to be stained so that the color of the tissue does not fade. The reason is that most of the dyes used in staining have basic properties and the color is intensified or made more permanent by an alkali, and the dissolved lime answers this purpose, as already noted. *

Where it is impracticable to use dehydrated alcohol containing lime or acetylene in solution, the alcohol may be further purified by subjecting it to distillation, thus getting rid of the dissolved impurities from the action of the commercial carbide which is rather impure. On account of the solubility of acetylene in alcohol there will be a small amount found in the distillate. This is removed by adding a little finely-powdered and dried silver and mercuric nitrate or mercuric oxide. These salts form compounds with the acetylene and on again subjecting the alcohol to distillation they are eliminated and the distillate is obtained pure and absolute. Mercuric oxide removes sulphur compounds which are liable to be present, since thioaldehydes are almost always formed as a result of the action of an acid on calcium carbide.

Pure absolute alcohol is described as absolutely odorless and the product prepared by this method comes as near to being as free from having any odor whatever, as any we have ever obtained by the methods hitherto described. This fact strongly emphasizes the purity of the alcohol made from carbide according to the directions we have given.

The essential reason why calcium carbide is more efficient for dehydrating purposes, than quicklime is that it acts upon the water

* Midland Naturalist, Vol. 1, p. 28.

in two stages: $CaC_2+H_2O=CaO+C_2H_2$; $CaO+H_2O=Ca(OH)_2$. In the first stage of the reaction acetylene and calcium oxide are formed; and the calcium oxide then unites with more water producing calcium hydroxide. Thus for every molecule of carbide used there are two molecules of water acted upon. Using ordinary unslaked lime we have an agent which is theoretically one-half as efficient as carbide and practically never even half as efficient, since its reaction is the same as the one taking place in the second stage when carbide is used, consequently it has the disadvantage of not being as active in uniting with water as is carbide, and since it absorbs a great deal of alcohol, thereby the distillation from the residue becomes very slow and incomplete.

In an experiment using two liters of alcohol having a specific gravity of 832 or about 90 per cent purity, we obtained 1400 cc impure alcohol such as could be used for dehydrating in methods of histological technique,—the only piece of apparatus used being an ordinary bottle containing the mixture. By distilling the residue or slag remaining after these 1400 cc of alcohol had been decanted, nearly 400 cc more of alcohol absolutely free from water was obtained, thus making the yield almost quantitative. To this distillate were added a few grams of dried mercuric nitrate and some silver nitrate. The mixture was then allowed to stand about a day in a warm place till all the acetylene and other compounds, which are formed due to the impurities in the carbide, were acted upon.

In order to be sure that all the acetylene has combined with the mercury and silver salts, some of the clear liquid was decanted and tested with a small portion of finely powered silver nitrate. Any precipitate or dark coloration which formed on standing indicated the presence of impurities, and necessitated the addition of more mercury and silver salts.

When all the impurities from the carbide were removed, the liquid, after decantation or filtration from the precipitate, was distilled and found to have a specific gravity of .7876 at 25° C. or a purity of 99.8 per cent. The distillate had hardly any odor or a very slight but pure ethereal one, and was practically free from any foreign substance whatever. It is always necessary to free the liquid from any of the precipitates of silver or mercury carbides formed by the action of the acetylene on the purifying salts, for there is danger of their exploding or decomposing when the last portions of the alcohol are distilled.

It required several days to effect the dehydration, and 550 grams of calcium carbide was used. The dehydration may, how-

ever, be accomplished in a much shorter time by boiling with an excess of carbide on a water bath, and using a reflux condenser. Absolute alcohol was made by this latter process in 10 hours.

It was found that the final distillate measured 1600 cc or the yield was equivalent to 89 per cent. of the theory. A larger yield could have been obtained if greater care had been used in obtaining the last traces of alcohol from the slag in the distilling vessel. This yield is much greater than that obtained by any other method now in general use that we have tried, and the ease in which the dehydration is accomplished as well as its completeness, make the carbide method the best one that we have been able to find.

Migration of Birds in St. Joseph County, Ind., from March 1 to May 30, 1909.

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The accompanying observations on the migration of birds was made at Notre Dame, Ind., and immediate vicinity, and unless otherwise stated the dates refer to their arrival.

The condition of the weather always affects the migration of birds northward. In a mild winter, the first arrivals appear here about the middle of February. In 1906, an exceedingly mild winter, I saw a flock of about a dozen robins on the 15th of February ; on the 12th of the same month I saw a song sparrow. The full tide of migration, however, seldom sets in for any species before the 1st of March.

During this month a cold wave will check the coming of the birds, making their arrival a week or two later than usual. The same holds good in April. Birds that generally arrive in the last week of April, such as the catbird, purple martin and Baltimore oriole, may not appear until after the 5th of May.

The present spring was notable for cold weather. The first week in March and April the weather was comparatively warm. The remainder of these months it was cool and some days cold. In May up to the 21st the temperature was over 70° only three days. On eight days it was below 60°. The lowest temperature in May was 32° on the 1st of the month. In the evening snow covered the ground.